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TITLE: Identification of Chromosome 18q Transcripts Lost in Breast Cancer

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14. ABSTRACT

A novel recurrent homozygous region of loss at 18q22.3 was detected in 50% of breast tumors by array comparative genomic hybridization. Since chromosomal regions exhibiting homozygous deletion are rare and are usually the site of tumor suppressor genes, the following hypothesis was proposed: Encoded within the region of homozygous deletion at 18q22.3 is a transcript whose loss plays a role in the development or progression of breast cancer. A custom oligonucleotide expression microarray covering the deleted region was designed and synthesized. RNA was isolated from primary human mammary epithelial cells (HMEC; Lonza), converted to fluorescently-labeled complementary RNA and hybridized to the microarray. An approximately 500 basepair sequence was found to be transcribed in breast epithelial cells. This is a novel transcript that has not been described previously. We confirmed this transcript with a quantitative PCR assay using normal breast epithelial cell cDNA. We also detected this transcript in brain, cervix, spleen and thymus tissues. To identify the full-length transcript, a brain cDNA library was screened.

15. SUBJECT TERMS
Breast cancer, chromosome 18, copy number alteration, microarray, transcript

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Table of Contents

Introduction

Previously, we detected genomic alterations at an 18q22.3 region in breast cancer specimens by array comparative genomic hybridization. This alteration was confirmed using fluorescence *in situ* hybridization, which showed a homozygous deletion of this region in 50% of breast tumors analyzed. The focus of this research is to determine if there are sequences within this region of deletion that are transcribed in normal breast epithelial cells. This is innovative research in that we are the first to observe the homozygous deletion of this region in breast cancer. In addition, we have shown that this region is homozygously deleted in prostate cancer specimens but not in cancers derived from 10 other organ sites. Regions of homozygous deletion are not commonly found; and these regions are often the site of genes important in tumor suppression. The search for transcribed sequences within the region of homozygous deletion has the potential to lead to the discovery of genes that when lost, play a role in the conversion of breast epithelial cells to cancerous cells.

Body

The research accomplishments for:

Task 1: Development of a custom oligonucleotide tiled microarray covering both strands of the region of chromosome 18 contained in the RPCI11-25L3 bacterial artificial chromosome clone and hy bridization of the array with labeled RNA from primary breast epithelial cells to identify transcripts encoded within the region.

A custom expression microarray consisting of 60-mer oligonucleotide probes tiled every 10 basepairs across the 185,000 basepairs region of deletion at chromosomal region 18q22.1 was designed and synthesized by Agilent Technologies (Santa Clara, CA). Total RNA was isolated from normal primary human mammary epithelial cells (HMEC; Lonza, Walkersville, MD). This RNA was converted to Cyanine-3-labeled complementary RNA and hybridized to the microarray using Agilent methodologies. The fluorescence intensity of the probes was analyzed using CGH analytics (Agilent) (Figure 1). We detected a contiguous region of hybridization to the microarray of approximately 500 basepairs. This is a novel transcribed sequence that has not been described or predicted previously. Using the NCBI Open Reading Frame Finder, there are two potential open reading frames of 30-40 amino acids in this transcribed sequence.

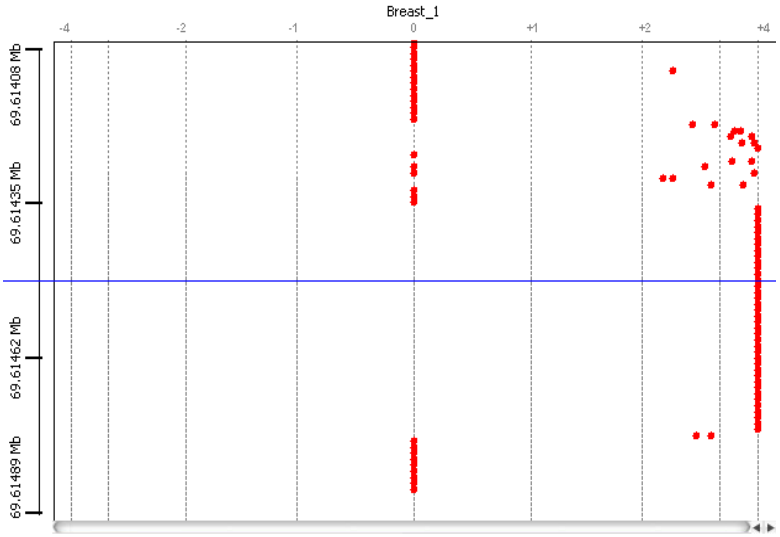

Figure 1.

Detection of a contiguous sequence transcribed in normal breast epithelial cells within the region of homozygous deletion. Each dot represents a probe. Dots to the right of zero indicate probes that hybridized with the labeled complementary RNA. The genomic positions of the probes are indicated on the y-axis.

There are other smaller regions of hybridizati on that may potentially be other transcribed exons (data not shown). There are six probes in the middle of this region that did not hybridize with the labeled complementary RNA. This may be due to the fact that the probes were chosen for the microarray based only on the criteria that the probes were tiled every 10 basepairs. The probes were not checked for optimal hybridization criteria, which will result in some probes showing less than optimal hybridization.

Task 1 is completed.

Task 2: Confirm and analyze transcripts identified in the region.

We designed a quantitative real-time TaqMan assay (Applied Biosystems; Foster City, CA) to verify the existence of this newly-discovered novel transcript in normal breast epithelial cells. An endogenous control assay to detect the levels of the large ribosomal phosphoprotein (RPLP0) was also used to normalize for the amount of cDNA in the assay. Total RNA isolated from primary breast epithelial cells and primary prostate epithelial cells was reverse transcribed into cDNA using the High Capacity cDNA kit (Applied Biosystems). We were able to detect the novel transcript in RNA isolated from both primar y breast and primary prostate epithelial cells. We have also used this TaqMan assay to determine if this sequence is expressed in a panel of RNAs derived from 20 normal human tissues (Ambion; Austin, TX ; Figure 2). The novel transcript was detected in highest levels in RNAs derived from brain (3), cervix (4), spleen (16) and thymus (18). This transcript is of relatively low abundance as detectable levels of PCR product were not detected much before 32 cycles of PCR.

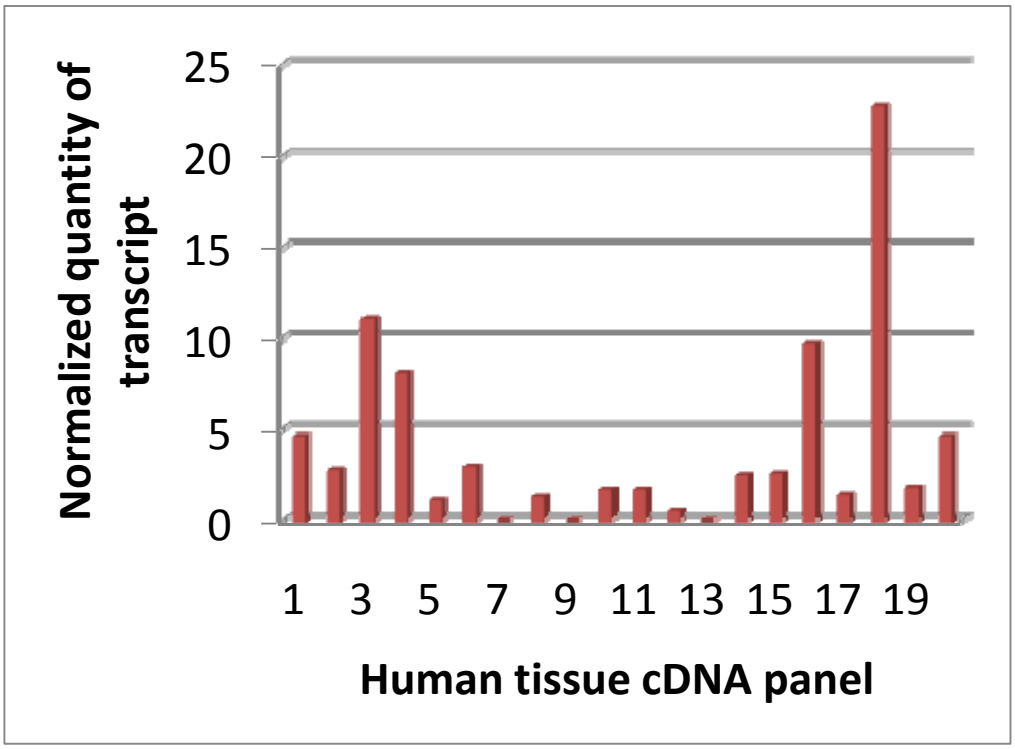

Figure 2. Real-time PCR was performed to determine the quantity of the novel transcript in a panel of human RNAs from various normal organs. The mRNA level of the novel transcript was normalized to the level of mRNA for the large ribosomal phosphoprotein RPLP0.

Our microarray experiment demonstrated that the novel transcript consisted of at least 500 basepairs. Our next goal was to isolate a full-length transcript. Since the transcript was expressed in normal brain tissue, we obtained a commercially available human brain cDNA library (OriGene Technologies; Rockville, MD) that is gridded for PCR analysis in order to screen for a full-length transcript. We screened the library and obtained positive clones, but upon analysis of the clones by sequencing we found these clones to be unrelated transcripts from a different chromosome, due to a probable PCR artifact. Since our quantitative PCR experiments indicated that the transcript is of low abundance, we believe that the transcript was not well represented in the particular cDNA library that we screened. Our alternative approach to assist us in identifying the full-length transcript is to use the rapid amplification of cDNA ends (RACE) technology. We have designed gene-specific primers and are currently performing RACE experiments with high quality RNA isolated from both brain and thymus.

Task 2. We confirmed the presence of the transcript in breast and prostate epithelial cell RNA and discovered that the transcript is detectable in RNA isolated from four other tissues.

Key Research Accomplishments

- **Recurrent homozygous deletion of a region at 18q22.3 w as found in 50% of breast tumors.**
- **Homozygous deletion of this region is specific to breast and prostate cancer and not cancers from 10 other organs.**
- **A novel transcript has been discovered in normal breast epithelial cells that is encoded in this region.**
- **A real-time PCR assay has been designed to quantitatively detect the level of the transcript.**
- **This transcript has been found in RNA isolated from normal human brain, cervix, spleen and thymus.**

Reportable Outcomes

Our results from this project were presented as a poster at the DOD Era of Hope meeting held in Baltimore, MD in June 2008.

Conclusions

Our discovery that a region of recurrent homozygous deletion in breast and prostate cancers encodes a novel transcript is significant. Recurrent homozygous deletions are relatively rare and indicate the presence of tumor suppressor genes. Although we were unable to isolate a full-length transcript during the funding period of this award we are continuing with the further characterization of this transcript. Continued characterization will help us to understand how loss of this transcript affects the process of breast cancer development and/or progression. In addition, we are currently determining whether this region encodes novel microRNAs. These new findings have the potential to lead to the identification of new targets for developing therapeutics.

Meeting Abtract

Era of Hope 2008 Meeting Abstract

Title: Detection of 18q Transcripts Deleted in Breast Cancer

Author(s):
Teresa L. Johnson-Pais; Fumika Matoba; Devon C. Hall; Susan L. Naylor and Robin J. Leach.

Presenter: Teresa L. Johnson-Pais

Array comparative genomic hybridization ex periments were performed on breast cancer specimens using genomic arrays covering the chromosomal region 18q21-q23. A novel homozygous region of loss at 18q22.3 was detected in 50% of breast tumors. Homozygous deletion of this region was also detected in prostate cancer, but not in cancers from 10 other organ sties. There are no known genes located within this region.

Since chromosomal regions exhibiting homozygous deletion are not commonly found and are usually the site of tumor suppressor genes, we proposed the following hypothesis: Encoded within the region of homozygous deletion at 18q22.3 is a transcript(s) that plays a role in the development or progression of breast cancer. In order to test our hypothesis, we proposed to: 1) Develop a custom oligonucleotide microarray covering both strands of this region; and hybridize the array with labeled cDNAs reverse transcribed from RNAs (large and micro) isolated from primary breast epithelial cells, and 2) Confirm and analyze the identified transcripts.

For aim 1, we have developed an oligonucleotide microarray tiled every 10 basepairs covering the 18q22.3 deleted region that is being synthesized by Agilent Technologies . RNAs (large and micro) have been isolated from the commercially-available human primary mammary epithelial cell line HMEC (Lonza) and converted to cDNA. The cDNA will be labeled and hybridized to the microarray. Aim 2 will confirm and characterize full-length transcribed sequences. Following the identification of transcribed sequences, commercially-available breast cancer cell lines will be analyzed to determine if the region is deleted and the expression level of the transcript(s) will be studied in these lines. Through these experiments we hope to identify a novel molecular pathway in breast cancer that could provide new targets for developing therapeutics.

List of Personnel Supported by Award

Teresa L. Johnson-Pais, Ph.D. – Principal Investigator
Stephanie A. DeMoor – Student Assistant